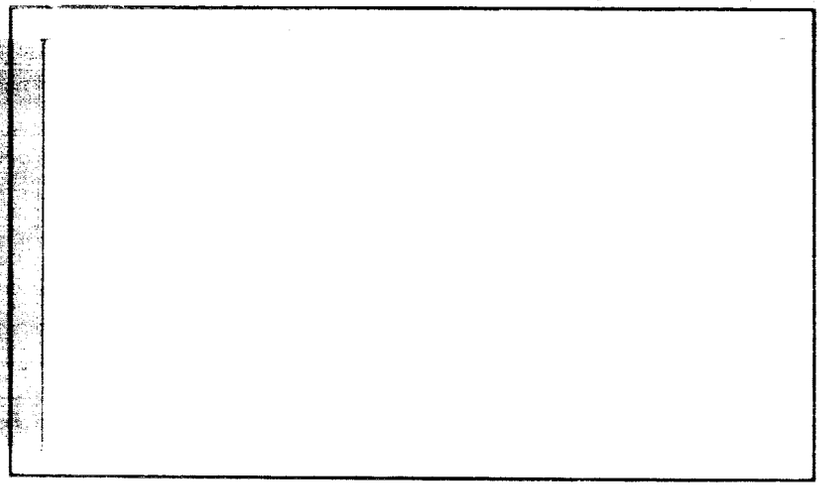

REPUBLIC
AVIATION CORPORATION

Quarterly Progress Report

Covering Period: April 1 - June 30, 1964

Study of the Normal Fecal Bacterial Flora of Man

RAC 931-4

Prepared Under Contract NASw-738

by

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INTRODUCTION

The work conducted this quarter has centered largely around the study of the physiology of many of the predominating type cultures isolated from the feces of the adult male subjects on this study. A new series of subjects has been started and will be discussed briefly.

PHYSIOLOGY OF TYPE CULTURES

Studies were continued on some fundamental biochemical capabilities of the anaerobic type cultures. Up to now the physiology studies have emphasized the utilization of two major classes of nutrients: amino acids and carbohydrates. This reporting period is primarily concerned with carbohydrate fermentation. It is significant because it is a beginning in an understanding of competitive removal of food from the intestine, ecological relationships between fecal bacteria, and taxonomy of the predominant fecal anaerobes.

Our approach to the study of carbohydrate fermentation was to start with glycolysis, since the anaerobic utilization of glucose is important taxonomically and in the intestinal ecology. Two techniques were used in the glycolysis studies:

1. Assay of lactic acid formation in a glucose-containing growth medium
2. Manometric determination of gas and acid by produced from glucose by resting cells.

Lactic Acid Production

A modification of the procedure of Hullin and Nobel (1953 Biochem. J. 55, 289.) was developed for lactic acid. It consists of the following steps:

1. The sample is first freed of bacterial cellular debris and unmetabolized proteins by centrifugation and precipitation with tungstic acid.

2. Glucose (the substrate) and pyruvic acid (a common end product of bacterial metabolism) are removed by triple extraction with copper sulfate and calcium hydroxide, since they interfere with step 4.

3. The solution is heated with concentrated sulfuric acid to convert lactic acid to acetaldehyde: an untreated control is run to determine background acetaldehyde.

4. Acetaldehyde from the previous step is reacted with parahydroxydiphenyl in the presence of copper catalyst. The resulting solution is read colorimetrically at 560 mu and compared to a standard lactic acid curve.

Samples were run in duplicate from each of two stationary phase cultures of bacteria. The arithmetic mean of the four determinations is listed in Table 1. The stock cultures are listed according to the percent lactic acid formed on a weight basis per unit weight of glucose. All tubes initially contained 0.1% glucose by weight: this quantity was chosen so glucose would be a limiting factor in the medium. Of course a complete carbon balance will require more comprehensive quantitative chemical analysis: this study was done only as a preliminary screening procedure to establish the relative quantities of lactic acid formed by the type cultures which were grown to the stationary growth phase on the same quantity of glucose. The purpose was to detect those microorganisms which have the capability to consume or form lactic acid.

The data in Table 1 illustrate several obvious points. FA-13 not only failed to produce lactic acid; it consumed lactic acid from the culture medium. The possibility of symbiotic lactic acid removal by FA-13 from a mixed culture containing a lactic acid producer could theoretically have ecological significance: autointoxication of the lactic acid producer could be prevented and the non-glucose ferventing symbiont could benefit by the lactic acid substrate.

Table 1 also illustrates that under the conditions of this experiment FA-4, FA-5 and FA-11 produced relatively large quantities of lactic acid, but FA-1, FA-3, FA-6 and FA-14 all produced relatively little. Another group FA-2, FA-9, FA-7, FA-8, FA-12 and FA-15 produced an intermediate amount.

Manometric Studies

The second part of the carbohydrate fermentation studies was concerned with manometric estimation of metabolic end products. By combining the results of manometric studies with the lactic acid production data, it was possible to group some microorganisms on the basis of their common characteristics and separate others on the basis of unique biochemical capabilities.

TABLE I

PERCENT CONVERSION OF GLUCOSE TO LACTIC ACID
BY TYPE CULTURES

Culture	% Lactic Acid/Weight Glucose
FA-13	*
FA-1	5
FA-14	9
FA-6	9
FA-3	9
FA-12	19
FA-15	21
FA-2	26
FA-9	26
FA-7	28
FA-8	28
FA-11	37
FA-4	39
FA-5	40

*These assays were 64% below the concentration of lactic acid in the uninoculated culture medium (18 mg/100 ml.).

Of the cultures studied, FA-13 has the following unique characteristics:

1. Morphology and staining
2. Lactic acid utilization
3. Inability to ferment glucose with the formation of either acid or gas

Both FA-4 and FA-5 have the following similarities:

1. Morphology
2. Lactose, maltose, and starch are fermented at much higher rates than glucose
3. Organic acids, but not measurable H_2 or CO_2 are formed when the substrates listed in (1) are fermented
4. Relatively high concentrations of lactic acid are formed in culture media

FA-14 had the unique capability of producing H_2 and CO_2, but no detectable organic acids from glucose. There were many rods that were homofermentative under the conditions of this experiment. Although FA-2 produced organic acids but no gas from glucose, it characteristically formed rods in chains. FA-15 was Gram negative. Three other homofermentative bacteria, FA-6 and FA-16, have similar glucose fermentation patterns and form acid, but not H_2 or CO_2 from glucose. However, the rate of growth on Gall's medium is different. FA-6 grows relatively well and FA-16 grows poorly. Although FA-11 is similar to FA-6 and FA-16, it has the additional capability of casein proteolysis.

Future work will consist of further analysis of the end products from glycolysis as well as amino acid utilization.

ISOLATION OF PREDOMINATING FECAL BACTERIA

A new group of ten young men has been selected as the second group of subjects and to date samples have been collected from six of these men, four of which have been processed as far as the keying of the anaerobes. Table 2 gives the results of the aerobic and approximate anaerobic counts on these six samples. With the exception of Subject No. 12 who had an unusually low recovery of anaerobes the results of these six subjects fall in line with the previous ten subjects studied in this program. Of the four subjects whose predominating anaerobes have been screen tested, three of the subjects gave results consistent with those found previously, since out of the total of seventeen

anaerobic cultures screened fourteen or 82% were keyable. The other subject who is of Italian extraction and eats a typical Italian diet of highly seasoned food gave an interesting result. Out of the seven anaerobes tested only two fell into the anaerobic key, but four of the five unidentified cultures appeared to be the same organism. The results are incorporated in Table 3. All of the cultures isolated from the top dilutions were obligate anaerobes with the exception of one facultative anaerobe found on Subject twelve, whose whole sample appeared to give unusual results.

Preliminary results from the initial sample on four young men tested off-site on another contract using similar procedures to those used in this study have been quite similar to those obtained on the study of men of comparable age at RAC.

TYPE CULTURES

The predominating anaerobic cultures which do not fit into the present key seem to be of rather wide variety with no one type of organism occurring often enough to warrant setting up a new type culture group at this time. However, the unusual organisms are being preserved so that if enough similar organisms occur to warrant setting up new group type cultures they can be included in the study.

PROJECT PERSONNEL

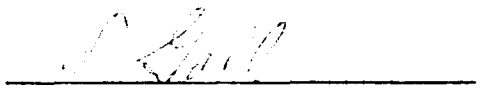
Personnel who have been working on the program are Dr. Lorraine S. Gall, Charles Huhtanen, Norman Richards, Raul Cardenas and Shirley Dunwoody.

HOURS EXPENDED: (April 1 - June 30, 1964)

Professional:	263
Technician:	424

PROJECTED WORK

During the next quarter it is intended to continue the physiology studies on the predominating anaerobes, to continue work with our second series of ten healthy young men and to set up new type culture groups if the need arises.



Lorraine S. Gall, Ph. D.

TABLE II

AEROBIC PLATE COUNTS AND
HIGHEST ANAEROBIC DILUTION GROWN

Subject	Aerobic In Millions	Highest Anaerobic Dilution Grown In Billions
12	50	10
15	235	10,000
16	90	1,000
17	500	1,000
18	600	1,000
19	35	1,000

TABLE III

NUMBER OF ANAEROBIC CULTURES SCREENED AND KEYED

Subject Nos.	Total Screened	Total Keyed
15	6	5
16	5	4
18	7*	2
19	6	5

* 4 cultures apparently the same